Laboratory Identification

of

Pathogenic Fungi Simplified

Publication Number 370

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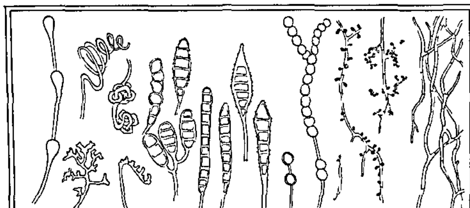
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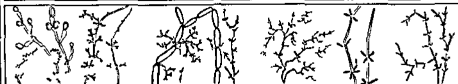
The BANNERSTONE DIVISION *of*

AMERICAN LECTURES IN TESTS AND TECHNIQUES

Edited by

GILBERT DALLDORF, M.D.





FRANK C. REED

(Second Edition)

Laboratory Identification of Pathogenic Fungi Simplified

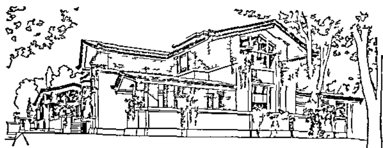
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To the Memory of

DR RHODA W BENHAM

1894–1957

who was a constant source
of inspiration and encouragement

Preface to the Second Edition

The objective of the second edition remains the same as that of the first—an aid in the teaching of the essentials in the identification of pathogenic fungi to the beginner and as a bench companion for the bacteriologist engaged in mycologic diagnosis

Many additions and substitutions have been made in the hope of increasing the usefulness of the monograph A section has been added on the contaminants commonly encountered in the laboratory and so often mistaken for pathogenic fungi by the inexperienced The colonial and microscopic characteristics by which these saprophytic fungi can be identified and differentiated from the pathogen are described and illustrated photographically

In the section on the pathogenic fungi incitants of South American blastomycosis aspergillosis mucormycosis and moniliasis incited by species of *Candida* other than *Candida albicans* are described South American blastomycosis is not endemic in North America but travel between the two continents is so common that the possibility of infection in persons who have been in South America should not be overlooked The systemic form of aspergillosis and mucormycosis has increased since the advent of the broad spectrum antibiotics steroids and folic acid antagonists

Drawings of characteristic microscopic structures of some of the common fungi constitute a new frontispiece which should be useful to the student in testing his ability to identify these fungi Some of the photomicrographs have been replaced with better prints Recent references have been included in the bibliography and new formulae have been added to the list of media

Again we acknowledge with deep appreciation the valuable assistance of our many colleagues without which this second edition would not have been possible

E L H
F C R

Preface to the First Edition

THIS MONOGRAPH is the outgrowth of an experience in teaching diagnostic mycologic methods to students who had been trained in bacteriologic procedures but who had little or no experience in the field of mycology Some students were assistants in this laboratory some were visitors from other laboratories and some were physicians preparing to direct local laboratories in New York State All wished to become familiar in a short time with the essential procedures and criteria in the identification of the incitants of fungus infections

As a visual aid in this program of instruction the exhibit panel shown in the *Frontispiece* was designed to present the characteristic features upon which the identification of the patho genic fungi is based Giant living colonies and diagrammatic drawings of the microscopic structures familiarized the students with the morphology of the fungi The exhibit was also used effectively in lecture demonstrations to medical students Later as a matter of convenience photographs of the individual sec tions of the exhibit were bound with a supplementary text as a manual for use at the laboratory bench Impetus to make the manual more widely available came from the response of mem bers of the New York State Association of Public Health Labora tories to whom it was shown at the Annual Meeting in 1952 In the present book photomicrographs replace the drawings of the original exhibit Tabular and other textual descriptive in formation has been added together with formulae of the essen tial culture media and a selective list of references The objective remains the same as in the earlier presentations namely an aid to the teaching of the essentials in the identification of the patho genic fungi to the beginner and a bench companion for the bacteriologist engaged in mycologic diagnosis

Only those pathogenic fungi commonly encountered in North America are included in this work and all illustrations are from cultures studied in this laboratory. The pathogenic fungi are, with few exceptions, members of the class of Fungi Imperfecti, that is, fungi in which the sexual spore has not been demonstrated. Identification is based upon asexual spores, the conidia, which are borne on specialized hyphae (conidiophores). The diseases caused by the pathogenic fungi have been roughly classified into superficial and systemic or deep seated mycoses.

In publishing this monograph our appreciation goes to the students whose responses have helped us to sharpen the various presentations. Acknowledgment is also made to our colleagues whose encouragement and valuable assistance are responsible for the decision to offer to others this outline of a practical laboratory experience.

E. L. H.
F. C. R.

Contents

Laboratory Identification

of

Pathogenic Fungi Simplified

Aided by a grant from the Brown Hazen Fund

Superficial Mycoses

Dermatophytoses (Ringworm)

THE SUPERFICIAL MYCOSES are the most common and widely distributed of all fungus diseases They are confined to the keratinized layers of skin and its appendages but nevertheless are of major importance since they are so widespread and may cause great discomfort and even at times are very disabling

These diseases are incited by a group of fungi the dermatophytes embracing many species The isolation and identification of the fungus from scrapings from skin lesions infected nails or from stubs of broken hairs are essential to specific diagnosis

This group of fungi is represented by three genera based upon the type of macroconidia (fuseaux) formed *Microsporum Trichophyton* and *Epidermophyton*

Note The dermatophytes are usually easily isolated on Sabouraud s glucose agar at room temperature however in cases of grossly contaminated specimens a selective isolation agar Mycosel (Baltimore Biological Laboratory Inc) should be used.

MICROSPORUM

THE GENUS *Microsporum* consists of three commonly recognized species *M audouini M canis* and *M gypseum* They attack hair and glabrous skin These fungi are the chief incitants of ring worm of the scalp (tinea capitis) among children in the United States The infected hair shows a sheath of spores in the form of a mosaic about the hair shaft (ectothrix type) and under filtered ultraviolet light (Woods lamp) there is a brilliant green fluores cence In the infected skin segmented branching mycelium is found

They form cottony or downy matted or powdery aerial my celium and vary in color from white to grayish white or buff to various shades of brown

They produce characteristic large thick rough or smooth walled multiseptate spindle shaped macroconidia (fuseaux) and small single celled clavate spores attached directly or to short sterigmata on the sides of the hyphae pectinate hyphae nodular bodies racquet hyphae and chlamydospores are also formed

MICROSPORUM AUDOUINI

Media for Development of Significant Characters

MACROSCOPIC	
Sabouraud's glucose agar	Flat grayish white velvety aerial mycelium (with button in center) rose brown pigment on under surface
Polished rice grains	No aerial mycelium brownish discoloration of grains

MICROSCOPIC	
Corn meal agar	Pectinate hyphae
Honey agar plus yeast extract (10 mg/ml)	Macroconidia microconidia
Incubated at 25° C	

Microsporum audouini

The species *Microsporum audouini* is of human origin and is the chief agent of tinea capitis among children in the United States This dermatophyte also attacks glabrous skin

Cultural and Microscopic Characteristics

The colony on Sabouraud s glucose agar is slow growing flat with a button in the center and a surface of grayish white closely matted velvety sometimes fluffy aerial mycelium occasionally showing radial foldings a rose brown pigment on the under surface

Microscopically large thick rough or smooth walled multi septate spindle shaped macroconidia (fuseaux) and numerous single celled clavate microconidia and pectinate hyphae may be seen The macroconidia can seldom be demonstrated on Sabour aud s glucose medium An enriched medium is required

MICROSPORUM AUDOUINI

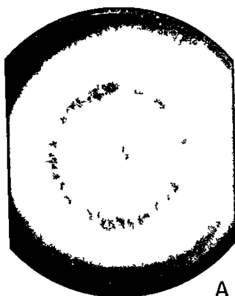
Figure 1

A Colony on Sabouraud's glucose agar after three weeks

B Honey agar plus yeast extract slide culture preparation showing multiseptate spindle shaped macroconidia and clavate microconidia X 400

C Culture mount from corn meal agar showing pectinate hyphae X 400

D Infected hair with small spores forming sheath around hair X 400


A


B


D


C



Microsporum canis

Media for Development of Significant Characters

MACROSCOPIC	
Sabouraud's glucose agar	White cottony or wooly aerial *mycelium becoming powdery* with age bright yellow to orange pigment on undersurface
Polished rice grains	Grains covered with heavy white cottony aerial mycelium becoming powdery with age pinkish buff discoloration of medium

MICROSCOPIC	
Sabouraud's glucose agar	Macroconidia microconidia
Polished rice grains	Macroconidia microconidia

Microsporum canis

(Microsporum lanosum Microsporum felineum)

The species *Microsporum canis* is of animal origin and is responsible for roughly 10 per cent of tinea capitis among children in the United States This fungus also attacks glabrous skin

Cultural and Microscropic Characteristics

The colony on Sabouraud s glucose agar develops fairly rapidly forming a white cottony or wooly aerial mycelium becoming powdery with a central depressed area sometimes showing radial folds The pigment on the undersurface is yellowish orange later changing to reddish brown

Microscopically numerous large thick rough walled multiseptate spindle shaped macroconidia (fuseaux) and numerous clavate or elongated microconidia are found Racquet hyphae and chlamydospores are also present

Microsporum canis

Figure 2

A Colony on Sabouraud's glucose agar after three weeks

B Slide culture preparation stained with lactophenol cotton blue showing numerous macroconidia and small single celled microconidia X 600

C Multiseptate spindle shaped macroconidia X 400

D Young macroconidia attached to hyphal branches X 400

E Racquet hyphae X 400


A


B


C


D


E

Microsporum gypseum

Media for Development of Significant Characters

MACROSCOPIC

Sabouraud's glucose agar	Powdery aerial mycelium of buff to light brown or cinnamon color
Polished rice grains	Grains covered with powdery aerial mycelium of pinkish cinnamon color

MICROSCOPIC

Sabouraud's glucose agar	Macroconidia
Polished rice grains	Macroconidia

MICROSPORUM GYPSEUM

(Microsporum fulvum)

THE SPECIES *Microsporum gypseum* long considered to be of animal origin has more recently been found to be a soil inhabiting fungus Of the three microspora it is least commonly encountered as the cause of tinea capitis or ringworm of the glabrous skin

Cultural and Microscopic Characteristics

The colony is comparatively fast growing shows a central cottony boss surrounded by a flat powdery cinnamon brown mycelium terminating in a border of downy white mycelium The pigment on the undersurface is reddish brown

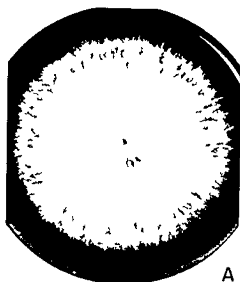
Microscopically numerous large rough thick walled multi septate spindle shaped macroconidia with slightly rounded ends are found

MICROSPORUM GYPSEUM

Figure 3

A Colony on Sabouraud's glucose agar after three weeks

B Numerous large rough thick walled multiseptate spindle shaped macroconidia with blunt ends X 400


A


B

TRICHOPHYTON

THE GENUS *Trichophyton* contains a large number of species which attack glabrous skin bearded areas hair and nails and causes a wide variety of lesions depending upon the species and the site of infection Infected hairs show either chains of spores outside the shaft (ectothrix type) or parallel rows of spores within the shaft (endothrix type)

Cultural and Microscopic Characteristics

These fungi form cottony velvety finely granular to powdery or glabrous aerial mycelium and vary in color from white to brown violet to purple pink to red or yellow to orange

The macroconidia (which cannot always be demonstrated in every species) are large clavate smooth thin walled multiseptate The microconidia produced in abundance in some species are single celled and are globular clavate or pyriform in shape and may be borne singly along the sides of the hyphae (en thyrse) or in grape like clusters (en grappe) Other structures which may be produced are spiraled hyphae nodular bodies racquet mycelium and chlamydospores Typical of some species of *Trichophyton* is the formation of the knobbed hyphal structures called favic chandeliers" as their only identifying characters

Trichophyton mentagrophytes

Media for Development of Significant Characters

MACROSCOPIC	
Sabouraud's glucose agar	Downy type—white, velvety to fluffy aerial mycelium; light buff to tan to brown on reverse surface. Granular type—white, powdery to granular aerial mycelium, sometimes pinkish; light buff to yellow to reddish brown pigment on reverse side. Cottony type—white, fluffy aerial mycelium; light buff to tan on reverse side.
Corn meal agar plus 1% glucose	No pigment on undersurface

MICROSCOPIC	
Sabouraud's glucose agar, wort, or corn meal agar	Microconidia, macroconidia, spirals, nodular bodies
Cottony type: Sabouraud's glucose agar, wort, or corn meal agar	Few or no microconidia

TRICHOPHYTON MENTAGROPHYTES

(Trichophyton gypseum)

THE SPECIES *Trichophyton mentagrophytes* includes many variants This dermatophyte is the chief incitant of the inflammatory type of ringworm involving the feet (athlete s foot) hands and glabrous skin particularly of the intertriginous areas The fungus also attacks nails bearded areas and the scalp causing the ectothrix type of hair

Cultural and Microscopic Characteristics

The variants of this species on Sabouraud s glucose agar show marked differences in colonial appearance the colonies having aerial mycelium ranging from cottony to powdery of white to cream to tan or pinkish color with cream to tan to reddish brown pigment on the reverse surface

Microscopically there are found in the downy and granular types numerous clavate to pyriform microconidia borne singly along the sides of the hyphae (en thyrse) and in clusters (en grappe) typical long clavate thin walled multiseptate macroconidia with constriction at septa Characteristic spiraled hyphae and nodular bodies may also be present In some of the variants particularly the fluffy or cottony forms no identifying structures or only a few microconidia may be found

Trichophyton mentagrophytes

Figure 4

Growth on Sabouraud's glucose agar after two weeks

A Cottony type

B Powdery to granular type

Microscopic structures—powdery type

C Stained slide culture preparation showing microconidia in clusters X 600

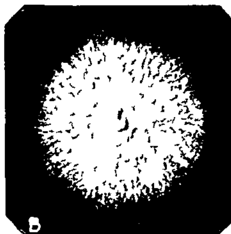
D Typical multiseptate clavate macroconidia X 400

E Culture mount from corn meal agar showing nodular bodies X 400

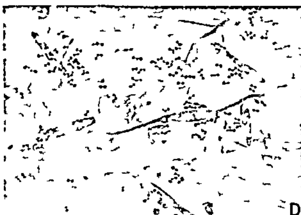
F Spiraled hyphae X 600

G Microconidia along sides of hyphae X 400


A

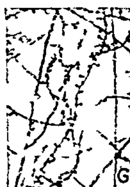

B


C


D


E


F


G

Trichophyton rubrum

Media for Development of Significant Characters

MACROSCOPIC	
Sabouraud's glucose agar	Cottony mycelium becoming velvety to powdery with central umbo reddish purple pigment on reverse side
Corn meal plus 1% glucose	Wine red pigment on undersurface

MICROSCOPIC	
Blood agar base (Difco)	Macroconidia microconidia

Trichophyton rubrum

(Trichophyton purpureum)

The species *Trichophyton rubrum* is the commonest incitant of chronic dermatophytosis of the hands and feet nails and glabrous skin the fungus may also involve the bearded areas including the hair follicles

Cultural and Microscopic Characteristics

The colony on Sabouraud s glucose agar at an early stage is white fluffy and hemispheric later becoming velvety to powdery with a central umbo and sometimes showing radial folds on the undersurface there is a characteristic reddish purple pigment

Microscopically long slender thin walled multiseptate macroconidia (fuseaux) with parallel sides and large numbers of pyriform microconidia borne from sides of hyphae are found For demonstration of the macroconidia a special medium is required

TRICHOPHYTON RUBRUM

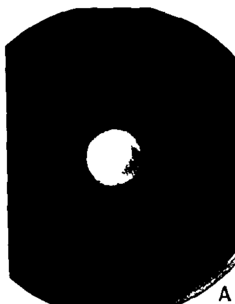
Figure 5

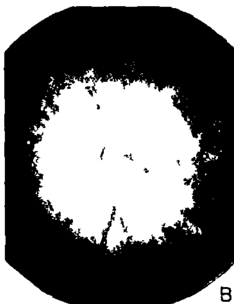
A Colony on Sabouraud's glucose agar after eight days

B Colony after three weeks

C and D Blood agar base slide culture preparations stained with lactophenol cotton blue showing microconidia along sides of hyphae and long, multiseptate macroconidia ×400

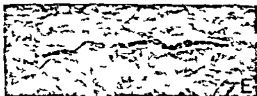
E Direct preparation from skin of patient with ringworm ×400


A


B


C


D


E

TRICHOPHYTON TONSURANS

Media for Development of Significant Characters

MACROSCOPIC	
Sabouraud's glucose agar	Heaped, irregularly folded, usually finely powdery aerial mycelium, creamy white to tan to yellow or shades of rose; on reverse dark brown pigment

MICROSCOPIC	
Sabouraud's glucose agar	Microconidia, irregularly branched hyphae, many chlamydospores
Polished rice grains	Macroconidia, microconidia

TRICHOPHYTON TONSURANS

(Trichophyton crateriforme)

THE SPECIES *Trichophyton tonsurans* attacks hair glabrous skin and nails The hairs show the endothrix type of infection

Cultural and Microscopic Characteristics

The culture is comparatively slow growing on Sabouraud's glucose agar The colony is heaped and folded sometimes showing a central crater or a central prominence with a surface of compact velvety or powdery aerial mycelium of creamy white tan yellow or shades of rose the pigment on the undersurface is dark brown

Microscopically there are large numbers of clavate or elongated microconidia attached singly to the sides of the hyphae or in loose clusters and few thin walled club shaped macroconidia long thick and irregularly branched hyphae and many chlamydospores

Trichophyton tonsurans

Figure 6

A Colony on Sabouraud's glucose agar after five weeks

B Sabouraud's glucose agar slide culture preparation stained with lactophenol cotton blue showing microconidia borne laterally on hyphae and in clusters X 400

C Growth from polished rice grains showing masses of microconidia X 600

D Growth from polished rice grains showing thin walled clavate microconidia X 400


A


B


C


D

Trichophyton verrucosum

Media for Development of Significant Characters

MACROSCOPIC	
Sabouraud's glucose agar Blood agar base plus thiamine	Glabrous heaped up cerebriform colony or disc shaped with white velvety mycelium or a much wrinkled glabrous colony of bright yellow color

MICROSCOPIC	
Blood agar base plus thiamine	Irregularly branched hyphae macroconidia microconidia chlamydospores
Polished rice grains	Macroconidia microconidia chlamydospores

TRICHOPHYTON VERRUCOSUM

(Trichophyton faviforme)

THE SPECIES *Trichophyton verrucosum* includes three varieties album discoides ochraceum This microorganism causes a suppurative type of ringworm involving the deeper layers of the skin and the hair follicles Infected hairs show large spores arranged in chains forming a sheath on the outside of the hairs (ectothrix type) and mycelial elements within the hair shaft Human infection is acquired directly or indirectly through contact with cattle having ringworm lesions The fungus may be isolated on Sabouraud's glucose agar but usually an enriched medium is required

Cultural and Microscopic Characteristics

The three varieties of *T verrucosum* differ markedly in colonial appearance varying from a glabrous heaped up cerebriform slightly yellowish colony (*T verrucosum* var album) to a flat disc shaped colony covered with a short white aerial mycelium (*T verrucosum* var discoides) or a much wrinkled glabrous flat colony of a bright yellow color (*T verrucosum* var ochraceum)

Microscopically there will be seen long multiseptate macroconidia with pointed or bulbous distal ends elongated pear shaped microconidia borne sessile along the sides of the hyphae or in terminal clusters irregularly branched hyphae with vast numbers of intercalary and terminal chlamydospores Special media are essential for demonstration of macroconidia

TRICHOPHYTON VERRUCOSUM

Figure 7

Colonies on blood agar base plus thiamine

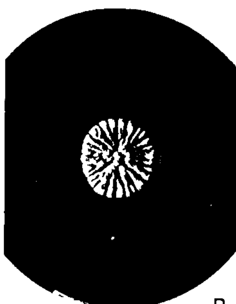
A *T. verrucosum* var. discoides after two weeks

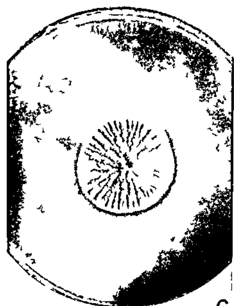
B *T. verrucosum* var. album after three weeks

C *T. verrucosum* var. ochraceum after four weeks

D Slide culture preparation stained with lactophenol cotton blue showing microconidia, clusters of microconidia and chains of chlamydospores ×400


A


B


C


D

Trichophyton schoenleini

Media for Development of Significant Characters

MACROSCOPIC	
Sabouraud's glucose agar	Glabrous, heaped and folded, sometimes showing a white powdery surface

MICROSCOPIC	
Sabouraud's glucose agar	Favic chandeliers, chlamydospores

Trichophyton schoenleini

(Achorion schoenleini)

The fungus *Trichophyton schoenleini* is the chief agent of favus a chronic fungus infection of the scalp and sometimes of the glabrous skin and nails The disease is rare in the United States In infected hairs large endothrix spores in chains are found together with mycelial elements and characteristic air bubbles The species can be isolated on Sabouraud s glucose medium but may be difficult to obtain in pure form due to bacterial contamination of the inoculum

Cultural and Microscopic Characteristics

The colony on Sabouraud s glucose agar is slow growing glabrous heaped and folded the surface later becoming covered with short white aerial mycelium a characteristic of the colony is the cracking of the medium

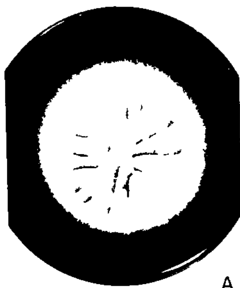
Microscopically the identifying structures are the favic chandeliers

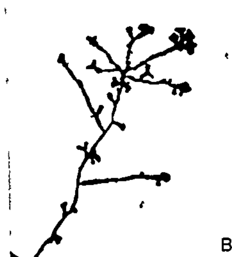
Trichophyton schoenleini

Figure 8

A Colony on Sabouraud's glucose agar after four weeks

B Sabouraud's glucose agar slide culture preparation showing "favic chandeliers" X 400


A


B

Trichophyton violaceum

Media for Development of Significant Characters

MACROSCOPIC

Sabouraud's glucose agar	Glabrous, heaped and folded, deep violet in color, sometimes showing fine white velvety mycelium on surface

MICROSCOPIC

Sabouraud's glucose agar	No identifying structures

TRICHOPHYTON VIOLACEUM

THE SPECIES *Trichophyton violaceum* attacks the hair of the scalp and bearded areas, the skin, and nails. The infection is found chiefly in immigrants in the United States and causes one of the most refractory types of ringworm of the scalp of all the trichophyta. The hairs show the endothrix type of infection, the large spores being arranged in rows within the hair shaft. The microorganism can be isolated on Sabouraud's glucose agar, but growth may not be recognizable for three to four weeks.

Cultural and Microscopic Characteristics

The colony on Sabouraud's glucose medium is moist, glabrous, heaped and folded, and shows a deep violet color, the surface at times becoming covered with a fine, white, short aerial mycelium.

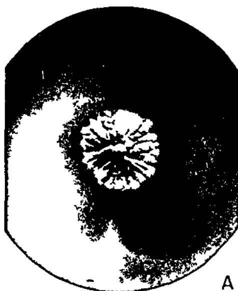
Microscopically, no identifying structures are found.

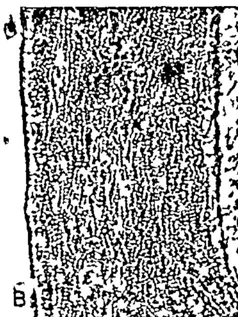
TRICHOPHYTON VIOLACEUM

Figure 9

A Colony on Sabouraud's glucose agar after four weeks

B Hair from ringworm of scalp showing endothrix spores X 600


A


B

EPIDERMOPHYTON FLOCCOSUM

Media for Development of Significant Characters

MACROSCOPIC	
Sabouraud's glucose agar	Velvety or felt-like surface with or without radial grooves, greenish yellow in color

MICROSCOPIC	
Sabouraud's glucose agar Corn meal	Macroconidia, chlamydospores

Epidermophyton

The genus *Epidermophyton* consists of a single species *E floccosum* (*T inguinale T cruris*) This dermatophyte attacks the skin particularly of the groin and the nails The hair is not affected

Cultural and Microscopic Characteristics

The colony on Sabouraud s glucose agar is moderately fast growing the surface is velvety or felt like often with radial furrows and an irregular folded center or the surface may be smooth the color is greenish yellow A characteristic of this culture is the appearance on its surface of small tufts of white fluffy aerial mycelium

Microscopically there will be seen the distinctive clavate macroconidia with blunt ends they are smooth thin walled and multiseptate and are frequently borne in banana like clusters numerous intercalary and terminal chlamydospores are also characteristic no microconidia are produced

EPIDERMOPHYTON FLOCCOSUM

Figure 10

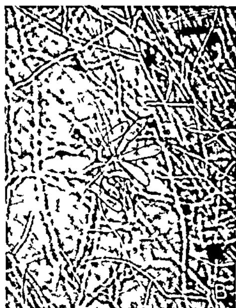
A Colony on Sabouraud's glucose agar after four weeks

B Cluster of multiseptate club shaped macroconidia on Sabouraud's glucose agar X 400

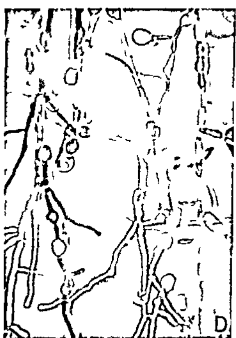
C Numerous typical club shaped macroconidia X 400

D Chlamydospores X 600


A


B


C


D

Deep-Seated Mycoses

(Subcutaneous Systemic)

ALMOST ALL THE deep seated mycoses have been encountered throughout the North American continent although the incitants are not necessarily endemic in all the areas where the diseases have been found Shifting populations and travel in endemic areas play a large part in the appearance of some of these diseases in areas where they are totally unsuspected As a consequence these infections frequently remain undiagnosed or are incorrectly diagnosed

These diseases which may be fatal or relatively benign are localized or systemic and are usually chronic but may be acute or subacute They are caused by a wide variety of fungi

These fungi attack the internal organs of the body bone meninges subcutaneous tissue skin and mucous membranes A definitive diagnosis cannot be established without the aid of the laboratory since demonstration of the fungus in pathologic specimens either by microscopic examination or cultural isolation is essential

A number of the most important fungi are described in the following pages under the specific diseases which they incite

Actinomyces bovis

Media for Development of Significant Characters

MACROSCOPIC	
Beef heart infusion agar with blood (anaerobic 7 to 10 days)	Colonies—white raised rough dry 1 mm or less in diameter with irregular surface and lobate edges
Beef infusion agar with 1% glucose in test tube* (aerobic incubation)	No growth for 5 to 15 mm below surface at upper level of growth definite band from 2 to 7 mm in width consisting of minute colonies below this many discrete colonies of variable size and of irregular shape
Beef infusion broth with 1% glucose (anaerobic 7 to 10 days)	Good growth clear supernatant flocculir or granular sediment tiny colonies up sides of tube

MICROSCOPIC	
Beef heart infusion agar with blood (anaerobic)	Gram positive branching filaments long and short rods coccoid forms
Beef infusion broth with 1% glucose (anaerobic 7 to 10 days)	Gram positive branching filaments pleomorphic rods coccoid bodies

ACTINOMYCES BOVIS

Figure 11

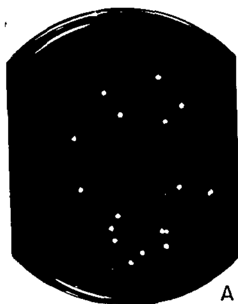
A Colonies on blood agar after two weeks incubation under anaerobic conditions

B Stained film preparation of culture from blood agar X 780

C Stained film preparation of pus from abscess in experimentally infected hamster X 600

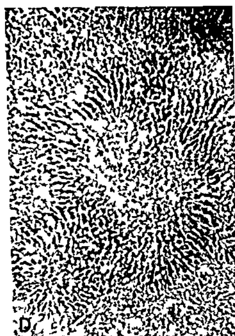
D Unstained granules with clubs in pus from encapsulated abscess in experimentally infected hamster X 600

E Section of encapsulated abscess showing granules with clubs and mass of filaments H & E X 900


A




C


D


E

Nocardia asteroides

Media for Development of Significant Characters

MACROSCOPIC	
Sabouraud's glucose agar slants (22-25° or 37° C.)	Raised, wrinkled, granular growth, ochre to yellow to orange in color, sometimes becoming covered with chalky white aerial mycelium
Gelatin	No liquefaction after 2 to 4 weeks at 22-25° or 37° C.
Milk containing bromcresol purple or litmus as indicator	Surface pellicle, no coagulation, no peptonization, alkaline reaction after 4 weeks at 22-25° or 37° C.

MICROSCOPIC	
Sabouraud's glucose agar	Gram positive, branching filaments, *fragmenting filaments, bacillary* and coccoid forms
Milk with indicator (3 weeks at 22-25° C.)	Acid fast, fragmenting filaments, bacillary and coccoid forms

NOCARDIOSIS

NOCARDIOSIS is a disease similar to or even clinically indistinguishable from actinomycosis The etiologic agent may be one of several species of the genus *Nocardia* N *asteroides* is the most commonly encountered species in the United States and is therefore described below

Specimens for Examination

Pus sputum cerebrospinal fluid biopsy specimens or post mortem material may be submitted

Cultural and Microscopic Characteristics

N asteroides is an aerobic Gram positive acid fast filamentous branching microorganism which breaks up readily into short rods or coccoid forms In sputum pus or other exudates it occurs in these forms In abscesses it may also be found in the form of granules with or without peripheral clubs The acid fast property of this species is relatively feeble as compared with the tubercle bacillus In culture this property can best be demonstrated by growing the microorganism in skim milk

The microorganism is easily isolated on Sabouraud s glucose agar at 22 25 or 37 C The colony on this medium is slow growing dry wrinkled and granular with a slightly heaped center and an irregular lobulate margin the color is at first whitish changing to ochre and later to orange sometimes becoming covered with a chalky white aerial mycelium In film preparations stained by Gram s method there will be seen long and short Gram positive branching filaments fragmenting filaments short bacillary and coccoid forms

Pathogenicity for Animals

The microorganism has a high degree of pathogenicity for the guinea pig

NOCARDIA ASTEROIDES

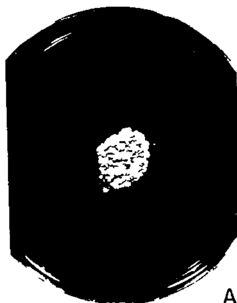
Figure 12

A Colony on Sabouraud's glucose agar after four weeks

B Stained film preparation of culture in milk showing acid fast filaments and rods X 1500

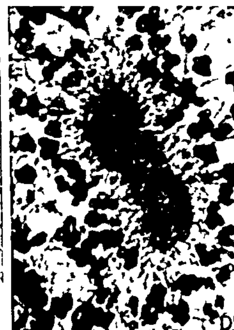
C Stained film preparation of pus from omentum of experimentally infected guinea pig showing fragmenting branching filaments X 600

D Section of guinea pig omentum showing abscess with central granule H & E X 600


A


B


C


D

Blastomyces dermatitidis

Media for Development of Significant Characters

MACROSCOPIC	
Blood agar (37° C.)	*Cream to tan, soft, wrinkled and waxy in appearance*
Sabouraud's glucose agar (22–25° C.)	White, fluffy aerial mycelium becoming cream to tan

MICROSCOPIC	
Blood agar (37° C.)	Large, round, single budding thick walled cells
Sabouraud's glucose agar (22–25° C.)	Round to pyriform microconidia borne laterally on hyphae

Blastomycosis

North American Blastomycosis (Gilchrist Disease)

This is a chronic disease which may be localized or systemic It is often localized in the skin forming granulomatous lesions with a verrucous surface and a raised smooth border sloping sharply to the normal skin The systemic form usually follows a primary pulmonary infection and may spread by the blood stream affecting any other organ or tissue of the body The etiologic agent is *Blastomyces dermatitidis*

Specimens for Examination

Bits of tissue from skin lesions pus from subcutaneous abscesses sputum bone blood or cerebrospinal fluid may be submitted

Cultural and Microscopic Characteristics

B dermatitidis exhibits dimorphism i e it grows in one form in tissue and in another form on culture media In pathologic material it appears as a large spherical thick walled budding cell of variable size 8 to 20μ in diameter on Sabouraud s glucose agar at room temperature it appears in the mycelial form

On blood agar medium at 37 C the growth is soft wrinkled and waxy in appearance cream to tan in color while on Sabouraud s glucose medium at room temperature the growth consists of white downy or fluffy aerial mycelium later becoming cream to tan to brown

Microscopically the culture on blood agar medium at 37 C appears in the same form as in tissue while on Sabouraud s glucose medium the growth consists of branched septate hyphae on which are borne small round to pear shaped conidia sessile or on short lateral branches

A culture thought to be *B dermatitidis* should not be identified as such without demonstration of its dimorphic nature

Pathogenicity for Animals

The microorganism is pathogenic for the young male hamster and to a lesser degree for the white mouse guinea pig and rabbit

BLASTOMYCES DERMATITIDIS

Figure 13

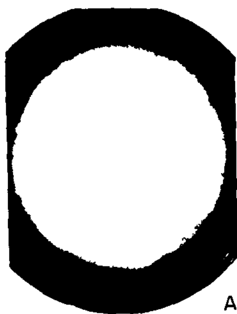
A Colony on Sabouraud's glucose agar after three weeks at room temperature

B Culture after 72 hours on blood agar at 37° C

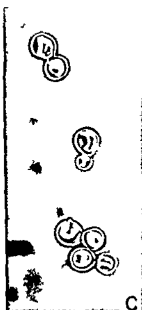
C Culture mount of growth on blood agar X 800

D Sabouraud's glucose agar slide culture preparation showing small round to pear shaped conidia X 400

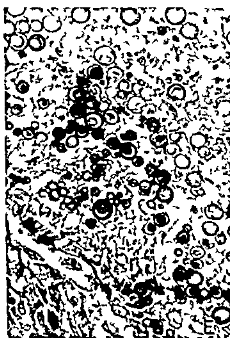
E Large spherical thick walled budding cells of variable size in lung tissue of man stained by Hotchkiss McManus technique X 400


A


B


C


D



BLASTOMYCOSIS

South American Blastomycosis (Lutz-Splendore-Almeida's Disease)

This is a chronic granulomatous disease of many manifestations depending upon the tissue involved. It may remain localized or become disseminated. Initial lesions, while commonest on the mucous membrane of the mouth and pharynx, may also be localized on the nasal or anorectal membrane, the skin, and in the lungs; frequently the larynx is infected from the lungs. Dissemination takes place by the hematogenous and lymphatic routes. The etiologic agent is *Paracoccidioides brasiliensis (Blastomyces brasiliensis)*.

Specimens for Examination

Bits of tissue from cutaneous or mucosal lesions, pus from lymph nodes or from cutaneous abscesses, sputum, bone, blood, biopsy material, or tissues from post-mortem examinations may be submitted.

Cultural and Microscopic Characteristics

P. brasiliensis is a dimorphic fungus. In pathologic materials the microorganism appears in the form of single and multiple budding, thick-walled, yeastlike cells, 10 to 60µ in diameter, and on artificial medium at room temperature in a mold-like or mycelial form.

On blood agar or chocolate agar at 37° C. the colonies are white, yeastlike, and cerebriform; on Sabouraud's glucose agar at room temperature the colony is compact, irregularly folded, and covered with a short, napped, white aerial mycelium, becoming brownish in old cultures.

Microscopically, the growth on blood agar at 37° C. consists of many single and multiple budding cells identical with the tissue forms, while on Sabouraud's glucose medium the growth at room temperature is composed of branched septate hyphae

A mycelial culture thought to be *P brasiliensis* should not be identified as such without conversion to the yeast phase and demonstration of multiple budding cells

Pathogenicity for Animals

The microorganism has been reported as pathogenic for the guinea pig rabbit hamster and white mouse

Paracoccidioides brasiliensis

Figure 14

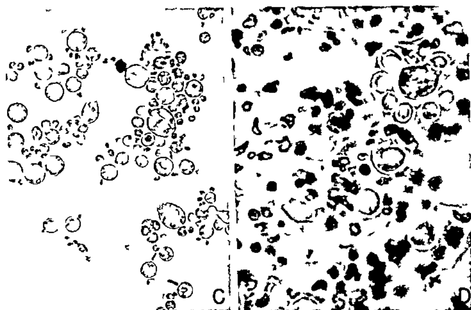
A Colony on Sabouraud's glucose agar after three weeks at room temperature

B Growth on blood agar after seventy two hours at 37 C

C Culture mount from blood agar at 37 C in lactophenol cotton blue X 600

D *Large spherical thick walled single and multiple budding cells in* human tissue H & E X 800 (Courtesy of Dr Margarita Silva Department of Dermatology College of Physicians and Surgeons Columbia University New York N Y)


A
B


C
D

Candida albicans

Media for Development of Significant Characters

MACROSCOPIC	
Sabouraud's glucose agar	Creamy, pasty, smooth

MICROSCOPIC	
Sabouraud's glucose agar	Round to oval budding cells
Corn meal agar dispensed in Petri plates	Mycelium, blastospores, chlamydospores

MONILIASIS (CANDIDIASIS)

MONILIASIS is a disease of many manifestations It may involve the skin particularly the intertriginous areas nails (onychia) and tissue around the nails (paronychia) the mucous membranes (mouth pharynx vagina) the perianal area broncho pulmonary system or even the blood stream or meninges The chief etiologic agent is *Candida albicans* other species which may cause infection are *C tropicalis C guilliermondi C parapsilosis C pseudotropicalis* and *C krusei C albicans* because of its greater importance is described in more detail A table with the differential characteristics of the six species is included

Specimens for Examination

Skin and nail scrapings exudates from mucous membranes sputum feces blood or cerebrospinal fluid may be required for examination

Cultural and Microscopic Characteristics

C albicans is a budding yeastlike microorganism which forms a true mycelium as well as pseudomycelium It appears in pathologic materials as budding cells and mycelial elements

It is easily isolated on Sabouraud s glucose agar The colony is cream colored pasty and smooth and has a yeastlike odor Microscopically there are found budding cells of varying shape which are of little aid in the specific identification of the microorganism

For identification the demonstration of the large round thick walled chlamydospore characteristic of *C albicans* is essential This may be accomplished by growing the microorganism on corn meal agar The corn meal agar dispensed in Petri plates should be inoculated with the primary culture by cutting through the agar along the line of streak and incubating at room temperature for several days Along the line of streaks *C albicans* develops mycelium on which is produced grape like clusters of blastospores and the round thick walled chlamydospores characteristic of this species

Pathogenicity for Animals

This fungus is pathogenic for mice and rabbits

Candida albicans

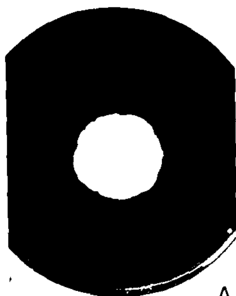
Figure 15

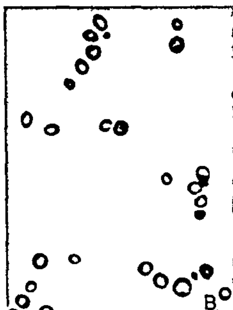
A Colony on Sabouraud's glucose agar after two weeks

B Culture mount from Sabouraud's agar X 800

C Culture mount from corn meal agar showing clusters of blastospores and large round thick walled cells (chlamydospores) along hyphae X 400

D Budding cells and mycelial elements in skin scrapings stained by Hotchkiss McManus technique X 400 (Preparation courtesy of Mr J Dennis Pollack of the Department of Dermatology College of Physicians and Surgeons Columbia University)


A


B


C


D

DIFFERENTIAL CHARACTERISTICS OF CANDIDA SPECIES*
Figure 16

Microorganism	Sabouraud's glucose agar culture	Sabouraud's glucose broth culture	Microscopic morphology corn meal	Sugar reactions D	M	S	I
1 *Candida albicans*	Cream colored pasty smooth colony or with mycelial border	Growth bottom of tube	Spherical or ball like clusters of blastospores along mycelium chlamydospores single or in grape like clusters at room temperature At 37 C no chlamydospores	AG	AG	A or O	O
2 *Candida tropicalis*	Cream colored membranous folded colony	Heavy growth at bottom of tube mucous like ring on surface	Blastospores single or in chains or irregular clusters anywhere along hyphal thread No or very few chlamydospores	AC	AG	AG	O
3 *Candida guilliermondi*	Like *C albicans*	No surface growth	Fine mycelium elongated cells clusters small	AC	O	AC	O
4 *Candida parapsilosis* (*C parakrusei*)	Cream colored pasty smooth colony	Growth bottom of tube no ring	Fine and coarse branched mycelium ie giant *forms* Blastospores single or in short chains or whorls from distal ends of cells	AG	O	O	O
5 *Candida pseudo tropicalis* (*C mortifera*)	Thin grey pasty colony	No surface growth	Elongated cells joined end to end readily separated blastospores not much differentiated from hyphal cells	AC	O	AG	AG
6 *Candida krusei*	Dull flat white colony	Definite film at surface	Elongated cells forming branched mycelium easily disintegrated Blastospores elongated in whorls at distal ends of hyphal cells	AG	O	O	O

* By per[illegible] Dr [illegible] (Rev [illegible])

Coccidioides immitis

Media for Development of Significant Characters

MACROSCOPIC	
Sabouraud's glucose agar	White cottony aerial mycelium becoming tan with age having a central zone of flat sparse mycelium

MICROSCOPIC	
Sabouraud's glucose agar	Coarse branching hyphae and chains of thick walled chlamydospores

COCCIDIOIDOMYCOSIS

COCCIDIOIDOMYCOSIS caused by *Coccidioides immitis* occurs in two forms—an acute self limiting respiratory infection (California disease valley fever) and a chronic sometimes fatal disease (coccidioidal granuloma) involving the cutaneous subcutaneous visceral and bony tissues

Specimens for Examination

Exudates from cutaneous lesions pus from abscesses sputum cerebrospinal fluid blood biopsy material or tissues from post mortem examination may be submitted

Cultural and Microscopic Characteristics

C immitis is dimorphic In the parasitic phase it appears as thick walled endosporulating spherules 5 to 50μ in diameter in different stages of development In culture at 37 C or at room temperature it occurs only in the mycelial form

The microorganism is easily isolated on Sabouraud s glucose medium or on Mycosel agar if the specimen is grossly contaminated The colony is moderately fast growing flat moist and membranous later developing a coarse white to tan to brown cottony aerial mycelium with central zone of flat sparse mycelium

Microscopically the culture shows coarse branching hyphae some broken up into thick walled spores chlamydospores usually in chains

Great precaution should be exercised when handling these cultures since the chlamydospores are easily dislodged and carried through the air Cultures on medium in Petri plates should not be employed Petroff flasks (22 x 95 mm) or tubes should be used instead

Pathogenicity for Animals

This fungus is pathogenic for white mice guinea pigs and rabbits The guinea pig injected intratesticularly with a suspension of the chlamydospores develops a severe orchitis within seven to ten days From such lesions pus may be aspirated and the typical endosporulating spherules demonstrated by direct microscopic examination

A culture thought to be *C immitis* should not be identified as such without demonstration of transformation in a susceptible animal of the chlamydospores to the characteristic parasitic growth phase (thick walled endosporulating spherules)

Coccidioides immitis

Figure 17

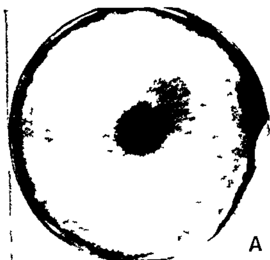
A Colony on Sabouraud's glucose agar after five weeks

B Culture mount showing chains of chlamydospores X400

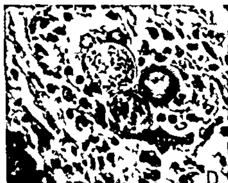
C Same X800

D Section from testicle of experimentally infected guinea pig, showing endosporulating spherules H & E X400

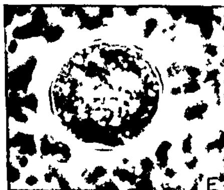
E Spherule X800


A


B


D


C


E

Cryptococcus neoformans

Media for Development of Significant Characters

MACROSCOPIC	
Sabouraud's glucose agar (22-25 and 37 C.)	Cream to tan in color, usually mucoid and glossy, occasionally dry and dull

MICROSCOPIC	
Sabouraud's glucose agar	Round budding cells of varying size surrounded by capsules of different diameters

Cryptococcosis

Cryptococcosis is a subacute or chronic infection chiefly of the central nervous system but may involve almost any part of the body The etiologic agent is *Cryptococcus neoformans* *(Torula histolytica)*

Specimens for Examination

Cerebrospinal fluid scrapings from superficial skin lesions pus from subcutaneous abscesses sputum bone lymph nodes blood bone marrow or specimens collected at post mortem may be submitted

Cultural and Microscopic Characteristics

Cryptococcus neoformans is a budding yeastlike microorganism In pathologic material and in culture it appears as a round occasionally oval budding cell of variable size 4 to 15μ in diameter surrounded by a refractile mucinous capsule The capsule varies in width sometimes being twice the diameter of the cell and can be demonstrated by mounting a loopful of the infected material or culture in a drop of India ink

The fungus is easily isolated on Sabouraud s glucose medium The colony is mucoid and slimy and is cream to brownish in color

The pathogenic strains grow readily at 22 25 and 37 C and do not assimilate KNO_3 while the nonpathogenic or avirulent strains grow poorly if at all at 37 C and assimilate KNO_3

Pathogenicity for Animals

The microorganism is virulent for white mice and rats For proof of the virulence of a given strain of *C neoformans* mice should be injected intravenously or intracerebrally and following the death of the animal budding cells with large capsules demonstrated in the brain

Cryptococcus neoformans

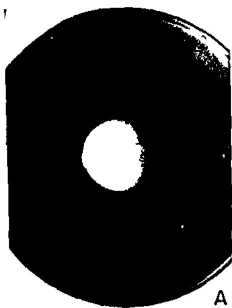
Figure 18

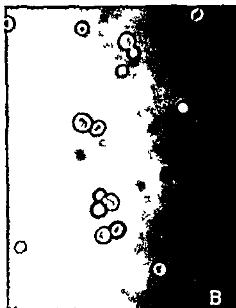
A Colony on Sabouraud's glucose agar after 10 days

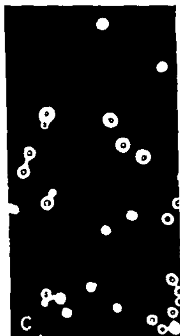
B Culture mount from Sabouraud's agar in drop of lactophenol ×450

C India ink preparation of culture from Sabouraud's agar ×400

D Section of spleen from white rat showing budding cells surrounded by capsules H & E ×525


A


B


C


D

Geotrichum candidum

Media for Development of Significant Characters

MACROSCOPIC	
Sabouraud's glucose agar	Flat membranous soft yeastlike consistency white to cream in color
Honey broth	White pellicle

MICROSCOPIC	
Sabouraud's glucose agar Corn meal agar	Chains of rectangular to round arthrospores

Geotrichosis

Geotrichosis is a comparatively rare and mild disease Lesions may occur in the mucous membranes of the mouth and intestinal tract in the bronchi and lungs The etiologic agent is *Geotrichum candidum*

This microorganism may be present on the skin in the mouth and in the feces of normal persons or as a secondary invader in pulmonary disease particularly tuberculosis A diagnosis of bronchial or pulmonary geotrichosis should therefore not be made without repeated demonstration of the presence of the fungus in the sputum by both microscopic and cultural examination and the elimination of other bronchopulmonary diseases

Specimens for Examination

Exudates from lesions in the mouth sputum or bloody stools may be submitted

Cultural and Microscopic Characteristics

G candidum appears in pathologic material as large rectangular to oval cells (arthrospores) and round thick walled non budding cells It can be easily isolated on Sabouraud s glucose agar

The colony on the Sabouraud s medium is flat membranous soft of yeastlike consistency white to cream in color and closely adherent to the medium A differential feature is the formation of a white pellicle in liquid medium

Microscopically it reveals broad septate mycelium and chains of rectangular to round arthrospores formed by fragmentation of the hyphae

Pathogenicity for Animals

Pathogenicity for laboratory animals has not been demonstrated

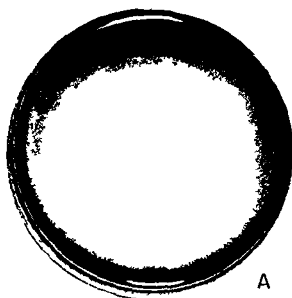
GEOTRICHUM CANDIDUM

Figure 19

A Colony on Sabouraud's glucose agar after three weeks

B Slide culture preparation on corn meal agar stained with lacto phenol cotton blue showing arthrospore formation X 400

C and D Same showing rectangular and round arthrospores X 800


A


B


C
D

HISTOPLASMA CAPSULATUM

Media for Development of Significant Characters

MACROSCOPIC	
Blood agar (37° C.)	Moist white yeastlike growth
Sabouraud's glucose agar (22-25° C.)	White fluffy aerial mycelium gradually becoming tan to brown

MICROSCOPIC	
Blood agar (37° C.)	Small oval budding cells
Potato glucose agar (22-25° C.)	Round to pyriform microconidia borne laterally on hyphae and large thick walled tuberculate chlamydospores (macroconidia)

Histoplasmosis

Histoplasmosis is a localized or generalized infection essentially of the reticuloendothelial system and may be acute sub acute or chronic involving almost any organ of the body The incitant is found chiefly as an intracellular parasite of the mononuclear cells in the peripheral blood sternal bone marrow lymph nodes or spleen The etiologic agent is *Histoplasma capsulatum*

Specimens for Examination

Tissue obtained by biopsy of lesions from skin mucous membranes and lymph nodes sputum gastric washings or tissue from post mortem examination including the brain may be submitted

Cultural and Microscopic Characteristics

H capsulatum is dimorphic In parasitized tissue it is found as small oval yeastlike cells 2 5μ in the longer diameter within the large monocytes on Sabouraud s glucose agar at room temperature it appears in the mycelial form

Colonies on blood agar at 37 C are small white yeastlike on Sabouraud s glucose agar at room temperature they are downy or cottony and white to cream later becoming tan to brown

Microscopically the culture on blood agar at 37 C occurs in the same form as in tissue while on Sabouraud s glucose agar it consists of septate hyphae on which are borne two types of spores (1) small smooth walled round to pyriform conidia sessile on the sides of the hyphae or on very short lateral branches (2) characteristic large thick walled spiny or tuberculate chlamydospores (macroconidia)

For the identification of a culture thought to be *H capsulatum* the demonstration of the dimorphic nature of the fungus as well as the presence of the tuberculate chlamydospores is essential

Pathogenicity for Animals

This fungus is pathogenic for mice young hamsters guinea pigs and rabbits

HISTOPLASMA CAPSULATUM

Figure 20

A Colony on Sabouraud's glucose agar after three weeks at room temperature

B Growth on blood agar after 72 hours at 37 C

C Growth from blood agar at 37 C in lactophenol X 100

D Culture from potato glucose agar showing small smooth walled round to pyriform conidia and tuberculate chlamydospores X 100

E Large thick walled tuberculate chlamydospores stained with lactophenol cotton blue X 1000

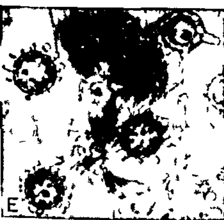
F Granulomatous lesion from ear of experimentally infected rabbit showing multinucleated giant cell filled with *H capsulatum* H & E X 1500


A


B


C


D


E


F

ASPERGILLOSIS

ASPERGILLOSIS is an inflammatory granulomatous infection. The skin, nails, external auditory canal, *paranasal sinuses, eye,* bronchi, bone, meninges, and brain may be involved. Several species of *Aspergillus* have been incriminated as agents of the disease: *A. fumigatus, A. flavus, A. nidulans, A. glaucus,* and *A. niger*. *A. fumigatus* is by far the most frequently encountered. These fungi are ubiquitous in nature and are commonly saprophytic; for reasons not understood they may become pathogenic and incite infection.

Pulmonary *aspergillosis as a primary infection is well recog*nized and occurs most often in agricultural workers, squab feeders, fur cleaners, and other persons in contact with fungus-contaminated grain. Its occurrence as a complication of bacterial infection of the lungs has become increasingly important with the widespread use of antibiotics, steroids, and folic acid antagonists. The portal of entry is usually the respiratory tract, from which the infection may become widely disseminated and terminate fatally.

Specimens for Examination

Sputum, skin, material from ear, and biopsy and post-mortem specimens may be submitted.

The disease is difficult to diagnose with certainty since the causative agent, usually *A. fumigatus*, is extremely widespread in nature and may appear in culture media or in pathologic specimens as a contaminant. The mere isolation or even the identification of the fungus is not sufficient. Demonstration of the fungus in tissue by direct microscopic examination is essential.

Cultural and Microscopic Characteristics

A. fumigatus is a filamentous fungus. In sputum or tissue it ap*pears as short, branching hyphal fragments, often with many small* (2 to 4μ) round green spores scattered throughout the field; occasionally the entire fruiting structure may be present. The *fungus is easily isolated on* Sabouraud's glucose agar at room

temperature or at 37 C Where there is marked bacterial con tamination streptomycin and penicillin should be added to this medium Medium to which cycloheximide (Actidione) has been added should not be used The colony is fast growing white at first soon becoming green or dark green flat and velvety The fungus grows well at 45 C or higher

Microscopically the identifying structure of this species as well as all species of *Aspergillus* is the conidiophore with the large terminal vesicle bearing many sterigmata (phialides) from which chains of spores are produced The conidiophore of *A fumigatus* is smooth walled and the conidial head columnar and compact with sterigmata in one series conidia are globose green and echinulate

Pathogenicity for Animals

The fungus is pathogenic to a high degree for the rabbit and to a lesser extent for the guinea pig

Aspergillus fumigatus

Figure 21

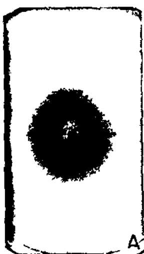
A Colony on Sabouraud's glucose agar seven days at room temperature

B Slide culture preparation on corn meal agar showing conidial heads X 600

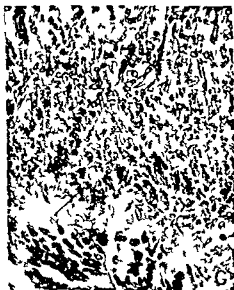
C Section from human lung showing septate hyphae H & E X 400

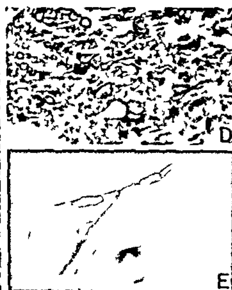
D Same section showing conidial head (arrow) X 800 (Courtesy of Dr J Lebowich Saratoga County Laboratory Saratoga Springs N Y)

E Hyphal fragment of *A fumigatus* in sputum (Reprinted with permission from Skinner C E Emmons C W Tsuchiya H M Henrici's Yeasts Molds and Actinomycetes 1947 John Wiley & Sons Inc)


A


B


C


D


E

Systemic Mucormycosis

Systemic mucormycosis is a disease characterized by vascular invasion with growth of hyphae, thrombosis, infarction, and reactive inflammation of hemorrhagic exudative and minimal granulomatous character. The infection may be incited by any one of several species of three genera (*Mucor*, *Rhizopus*, and *Absidia*) of the order *Mucorales* of the class *Phycomycetes*. The disease is being seen with increasing frequency, the pulmonary and cerebral tissues being most commonly attacked, often with a fatal outcome. These fungi may attack other tissues such as orbital, nasal, and paranasal sinuses and intestine and produce relatively mild unrecognized infection. From such sites dissemination may occur with a rapidly fatal result.

These *Phycomycetes* are ubiquitous and usually saprophytic but under certain conditions apparently have the capacity to incite disease. Their spores become airborne and enter the nose, sinuses, and lungs by inhalation or the gastrointestinal tract on contaminated food.

There are several predisposing factors: acidotic diabetes mellitus, leukemia, and other debilitating diseases; the use of steroids, folic acid antagonists, and prolonged antibiotic therapy is also believed to enhance susceptibility.

Specimens for Examination

Materials from sinuses, sputum, pus, cerebrospinal fluid, and biopsy and post mortem tissues may be submitted.

The laboratory diagnosis of mucormycosis is not easy and should be made only under carefully controlled conditions, since the etiologic agents are extremely widespread in nature and may appear as contaminants on media or in pathologic specimens. Demonstration of broad nonseptate hyphae by direct microscopic examination of pathologic specimens and isolation and identification of the fungus are essential for a definitive diagnosis.

Cultural and Microscopic Examination

The *Mucorales* are nonseptate filamentous fungi. In sputum

pus or tissue they appear as wide (limits of 4 to 50μ) nonseptate branching hyphal fragments occasionally the entire fruiting structure may be found They are easily isolated at room temperature on Sabouraud s glucose agar with the addition of streptomycin and penicillin or at 37 C on blood medium with antibiotics The colony grows rapidly quickly filling a Petri plate or test tube with fluffy mycelium first white later dark gray

Microscopically the hyphae are wide colorless and coenocytic The identifying structures are the single or branched sporangiophores which arise from any point on the hyphae or opposite rhizoids and bear large terminal globose spore filled structures the sporangia (See pages 132 133)

Pathogenicity for Animals

The *Mucorales* have only slight if any pathogenicity for the normal rabbit Cerebral mucormycosis has been produced experimentally in rabbits with alloxan induced diabetes by intranasal instillation of spore suspensions of these fungi

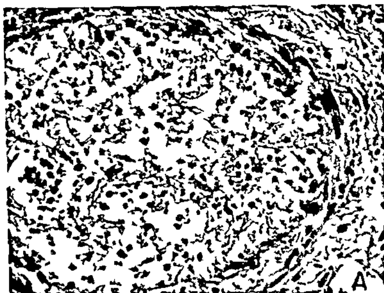
Agent of Mucormycosis

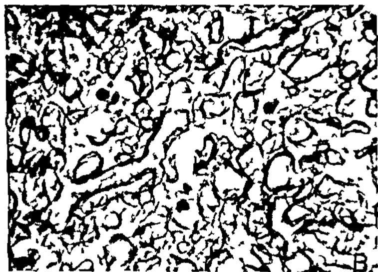
Figure 22

Section of human lung showing

A Wide nonseptate hyphae in pulmonary artery H & E X300

B Same in lumen X700


A


B

MONOSPORIUM APIOSPERMUM

Media for Development of Significant Characters

MACROSCOPIC	
Sabouraud's glucose agar	White cottony aerial mycelium becoming gray and spreading rapidly over entire surface of media blackened undersurface

MICROSCOPIC	
Corn meal agar	Light brown single ovoid conidia at tips of long or short conidiophores occasionally on the sides of hyphae

MADUROMYCOSIS

MADUROMYCOSIS is a chronic granulomatous disease usually involving only one of the lower extremities characterized by enlargement and deformity multiple draining sinuses and bone destruction The material from discharging sinuses generally contains granules (grains) irregular shaped masses of various colors white yellow red or black Among the incitants are species of *Madurella*, *Aspergillus* *Cephalosporium* *Allescheria* and *Monosporium* *M apiospermum* one of the chief incitants is described below

Specimens for Examination

Discharges from fistulae material aspirated from deep cutaneous and subcutaneous nodules may be submitted

Cultural and Microscopic Characteristics

M apiospermum is a filamentous fungus In pathologic material the fungus appears in the form of yellowish white granules lobulated structures composed of wide septate hyphae with numerous peripheral chlamydospores

It is readily isolated on Sabouraud s glucose agar at room temperature The colony on this medium is fast growing white cottony gradually becoming gray and has a grayish to black pigment on the undersurface

Microscopically there will be seen wide septate hyphae and large round oval or clavate conidia borne singly at tips of short or long conidiophores

M apiospermum is the imperfect form of *A boydii* also an etiologic agent of maduromycosis *A bodyii* produces asexual spores identical with *M apiospermum* for its identification the demonstration of perithecia (ascocarps) large (20 150μ) spherical brown to black structures is essential (See Figure 22C)

Pathogenicity for Animals

The pathogenicity of these species for small laboratory animals has not been established

Monosporium apiospermum

Figure 23

A Colony on Sabouraud's glucose agar after five weeks

B Culture mount from corn meal agar stained with lactophenol cotton blue showing single terminal conidia on long and short conidiophores X400

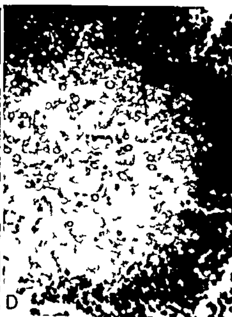
C Culture mount of *Allescheria boydii* from Sabouraud's glucose agar eight weeks showing young and mature ascocarps note large ripe ascocarp discharging ascospores X800

D Granule from experimentally infected hamster H & E X800


A


B


C


D

Sporotrichum schenckii

Media for Development of Significant Characters

MACROSCOPIC

Glucose cystine blood agar (37° C.)	Soft, yeastlike growth
Sabouraud's glucose agar (22–25° C.)	Leathery, wrinkled and folded, tan to brown to black

MICROSCOPIC

Glucose cystine blood agar (37° C.)	Round, oval, fusiform budding cells
Corn meal agar (22–25° C.)	Fine, septate hyphae and pear-shaped microconidia borne terminally in rosette-like clusters on lateral branches or singly along the hyphae

SPOROTRICHOSIS

THIS DISEASE is primarily a chronic infection of the skin and subcutaneous tissues characterized by nodular lesions which spread along the regional lymphatics generalized infections do occur but are rare in the United States The localized lesions are most frequently found on the extremities particularly the hands The etiologic agent is *Sporotrichum schenckii (S beurmanni)*

Specimens for Examination

Pus from subcutaneous nodules or from ulcerating lesions may be submitted

Cultural and Microscopic Characteristics

S schenckii is dimorphic In pus and necrotic tissue from human infection this microorganism appears as single-celled cigar shaped bodies usually within the polymorphonuclear leucocytes (these structures are extremely difficult to demonstrate in pus or tissue sections from human lesions) on Sabouraud's glucose agar at room temperature it grows in the mycelial form

On glucose cystine blood agar at 37 C the growth is creamy white soft and yeastlike On Sabouraud's glucose medium at room temperature the microorganism forms a mycelial colony at first white and soft later becoming tan to brown to black and leathery in texture and having a convoluted center from which extend radial grooves terminating in a flat border The appearance of the colony on this medium is diagnostic

Microscopically the culture on the blood medium at 37 C is found in the same form as in tissue while on Sabouraud's glucose medium the culture shows a fine branching septate mycelium and many small pear shaped conidia The conidia are borne terminally in rosette like clusters on lateral branches or are attached singly along the hyphae

Pathogenicity for Animals

The fungus is infectious for white male rats and mice and

SPOROTRICHUM SCHENCKII

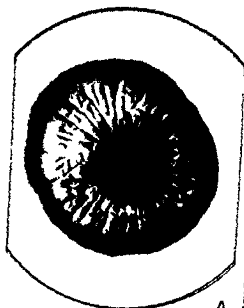
Figure 24

A Dark brown to black colony on Sabouraud's glucose agar after three weeks at room temperature

B Culture mount of growth from glucose cystine blood agar at 37 C X400

C Culture mount from corn meal showing fine branching hyphae and pear shaped conidia borne in rosette like clusters at tips of lateral branches (conidiophores) and singly along sides of hyphae X600

D Section from testicle of experimentally infected hamster showing Gram positive cigar shaped bodies Gram Weigert X800


A


B


C


D

Agents of Chromoblastomycosis

MACROSCOPIC (On Sabouraud's glucose agar)	
Phialophora verrucosa *Cladosporium carrionii* *Fonsecaea pedrosoi*	Colonies slow growing dark green to black felt like aerial mycelium
F compacta	Extremely slow growing
F dermatitidis	Black yeastlike at first gradually becoming filamentous at the periphery

MICROSCOPIC (On corn meal or Czapek's agar)	
Phialophora verrucosa	*Phialophora* type of sporulation only cup shaped conidiophores with spores that bud from the base of the cup
Cladosporium carrionii	*Cladosporium* type of sporulation only tree like branching conidiophores with conidia in abundance
Fonsecaea pedrosoi *F compacta* *F dermatitidis*	3 types of sporulation single or combined in one head (a) *Cladosporium* (b) *Phialophora* and (c) pseudo-*Acrotheca* conidia borne laterally on short protuberances on conidiophore *F compacta* differs from *F pedrosoi* in its broad based conidia *F dermatitidis* in its yeast like and *Pullularia* phases

Chromoblastomycosis

Chromoblastomycosis caused by several species of dematiaceous fungi was defined by Dr A L Carrion (1947) as a chronic infectious apparently non contagious skin disease confined most frequently to one of the lower extremities and characterized clinically by the formation of nodular verrucous or tumor like lesions More recently evidence of systemic disease has been reported There are at least five species representing three genera which may cause this disease *Phialophora verrucosa Fonsecaea (Hormodendrum) pedrosoi F compacta F dermatitidis* and *Cladosporium carrionii*

Specimens for Examination

Crusts or exudates from the verrucous lesions may be submitted

Cultural and Microscopic Characteristics

These fungi are dimorphic In tissue pus or caseous material they are indistinguishable They appear as large round yellowish brown thick walled often septate bodies On Sabouraud s glucose medium they are filamentous at 22 25 and 37 C

The colonies on this medium are slow growing dark green to black felt like in appearance with the exception of *F dermatitidis* and slightly embedded in the substrate They cannot be differentiated by study of macroscopic characters alone due to their striking cultural similarity *F dermatitidis* is at first yeast like gradually becoming mycelial at the periphery

Species identification is based upon the method of sporulation of which there are three types *Phialophora* characterized by cup shaped conidiophores with spores which bud from the base of the cup *Cladosporium* by tree like branching conidiophores

with conidia in chains *Acrotheca* by swollen conidiophores with lateral protuberances from which are borne singly ovoid conidia These three types of sporulation may occur simultaneously in a culture *F dermatitidis* in addition to the three types of sporulation also shows at a very early stage yeastlike and *Pullularia* phases

P verrucosa exhibits the *Phialophora* type of sporulation only

F pedrosoi the most common incitant of chromoblastomycosis possesses a triple sporulating ability All three types of sporulation occur simultaneously and any one may predominate

F compacta exhibits the three types of sporulation sometimes all combined in a single spore head with the *Cladosporium* type predominating

F dermatitidis exhibits early yeastlike and *Pullularia* phases and later reveals in addition the three types of sporulation characteristic of the genus *Fonsecaea*

C carrionii shows the *Cladosporium* type of sporulation only The pathogenic strains must be differentiated from saprophytic species of this genus on the basis of cultural characters The pathogens grow at 22 25 and 37 C and are slow growing whereas the saprophytic forms are fast growing and do not grow at 37 C

Pathogenicity for Animals

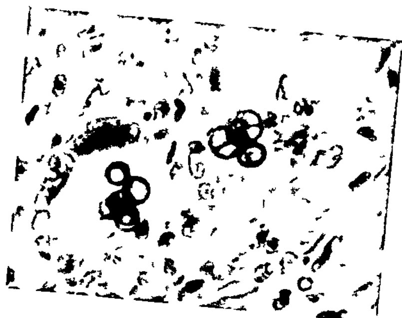
Rats and mice appear to be susceptible to these fungi injected by the intraperitoneal or intratesticular route

Grateful acknowledgment is made to Dr Margarita Silva for her assistance in the preparation of this section

Chromoblastomycosis

Figure 25

Histopathologic section from lesion in chromoblastomycosis showing large septate bodies X 800 (Courtesy of the late Dr Rhoda Benham Department of Dermatology College of Physicians and Surgeons Columbia University)



PHIALOPHORA VERRUCOSA
FONSECAEA PEDROSOI
FONSECAEA DERMATITIDIS

Figure 26

Phialophora verrucosa

A Colony on Sabouraud's glucose agar, four weeks

B Cup-shaped conidiophores with spores ×400

Fonsecaea pedrosoi

C Colony on Sabouraud's glucose agar, five weeks

D-F Three types of sporulation

D *Cladosporium* ×600

E *Acrotheca* ×600

F *Phialophora* ×600

Fonsecaea dermatitidis

G Colony on Sabouraud's glucose agar, 25 days

H Yeastlike phase, note double budding cells ×800

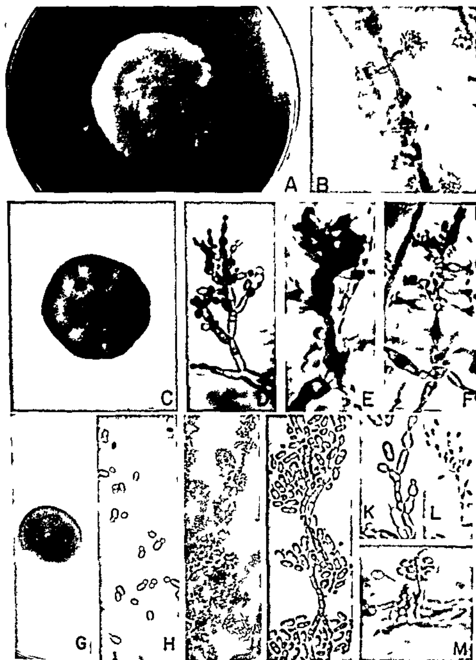
I *Pullularia* phase, showing clusters of spores along hyphae ×200

J Same ×800

K *Cladosporium* type of sporulation ×800

L Same ×600

M *Acrotheca* type of sporulation ×800


A
B
C
D
E
F
G
H
K
L
M

FONSECAEA COMPACTA
CLADOSPORIUM CARRIONII

Figure 27

Fonsecaea compacta

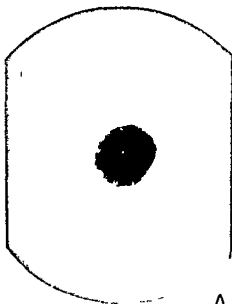
A Colony on Sabouraud's glucose agar, six weeks

B Complex conidial head, X 400

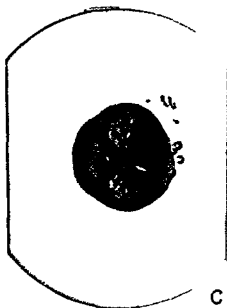
Cladosporium carrionii

C Colony on Sabouraud's glucose agar, four weeks

D Cladosporium type of sporulation, X 400


A


B


C


D

CONTAMINANTS

SAPROPHYTIC FUNGI are a constant nuisance in laboratories engaged in medical mycologic work. They appear frequently on the usual plating media, are often found in pathologic materials and are at times mistaken for pathogenic fungi. To learn to differentiate between the saprophytic and the pathogenic fungi is a fundamental requirement in the laboratory attempting mycologic examinations if serious mistakes are to be avoided in the diagnosis of fungus disease.

Recognition of contaminants should not be difficult provided one is familiar with a few of their common characteristics. The saprophytic fungi usually grow well at room temperature on the common mycologic media and poorly or not at all at 37° C.; they are in general highly pigmented, forming bluish green or green, cream to yellow, orange, brown, deep rose or black colonies, and nearly always produce an abundance of characteristic reproductive structures. The pathogenic fungi, on the other hand, are commonly slow growing at room temperature or at 37° C. and often require special media for spore formation. There are some pathogenic fungi that form dark green or black pigments, and some species of contaminants that are white or light colored. Therefore, a fungus isolated from a pathologic specimen should not be discarded as a saprophyte because of its pigment without a thorough cultural examination and microscopic study; neither should an isolate because it is white be considered a pathogen without an equally thorough study.

Eighteen common contaminants encountered in the laboratory are described and illustrated in the following pages. The giant colonies of all these fungi were grown on Sabouraud's glucose medium at room temperature. Classification by genus is adequate in the diagnostic laboratory for the identification of contaminants. Photomicrographs to illustrate the microscopic structural characteristics by which these contaminants can be identified generically are presented. These structures are best demonstrated in slide culture preparations on Czapek's or corn meal agar

PENICILLIUM SP
PAECILOMYCES SP

Figure 28

Penicillium sp

A Colony fast growing attaining a diameter of 52 mm in ten days flat with a powdery bluish green surface surrounded by a narrow white border

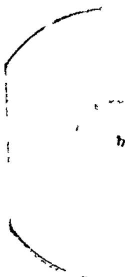
B Microscopically the diagnostic structure known as the "penicillus" (a) or brush is seen This structure consists of chains of spores pinched off from flask shaped sterigmata (phialides) (b) borne in whorls from the ends of metulae (c) (short branches) arising from branched or unbranched conidiophores (d)

> Many species of this genus differ widely in color texture and rate of growth Generic identification must be based upon demonstration of the "penicillus" or brush

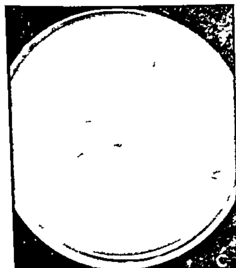
Paecilomyces sp

C Colony rapidly growing attaining a diameter of 60 mm in five days flat with a surface of yellowish brown powdery mycelium

D Microscopically the conidial bearing structures (a) suggest *Penicillium* but differ in that the flask shaped sterigmata (b) are long and tubular bend away from the axis of the conidiophore (c) and are not always in verticils (whorls) Long well separated chains of small elliptical conidia (d) are borne at the tips of the sterigmata




b
c
d
a
c
A
B


C


c
b
d
b
a
D

SCOPULARIOPSIS SP.
CEPHALOSPORIUM SP.

Figure 29

Scopulariopsis sp.

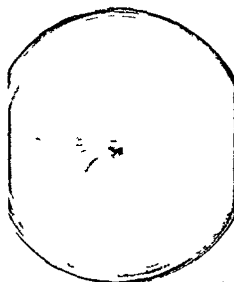
A. Colony fast growing, attaining a diameter of 65 mm. in eleven days; flat, at first white, later light brown and powdery with a light tan periphery; undersurface brownish at center fading gradually to a light tan.

B. Microscopically, the hyphae bear many short simple or branched conidiophores (a) which suggest the "penicillus" of *Penicillium*. The sterigmata (b) produce chains of lemon-shaped conidia (c) with a somewhat pointed apex and a truncate base. The mature spores are echinulate.

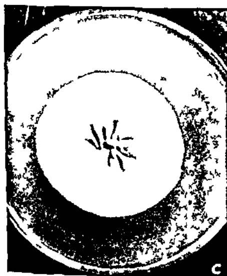
Cephalosporium sp.

C. Colony moderately fast growing, attaining a diameter of 51 mm. in seventeen days; compact, center slightly depressed, irregularly folded, cream colored, felt-like and surrounded by a slightly raised, pure white border of finely powdery mycelium terminating in a thin long-napped mycelium; reverse, pale yellow.

D. Microscopically, numerous elongate hyaline conidia borne in spherical clusters (a) at the tips of long or short lateral conidiophores (b).


A


a
b
c
c
B


C


a
b
D

TRICHOSPORON SP
RHODOTORULA SP

Figure 30

Trichosporon sp

A Colony moderately fast growing, attaining a diameter of 40 mm in thirteen days; ivory, membranous and radially folded; center moist and yeastlike.

B Microscopically, pseudomycelium and true mycelium are formed and many blastospores (a) and arthrospores (b).

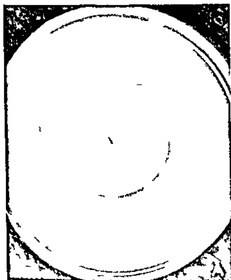
> This fungus is often found as a contaminant in sputum. Formation of blastospores differentiates it from *Geotrichum*.

Rhodotorula sp

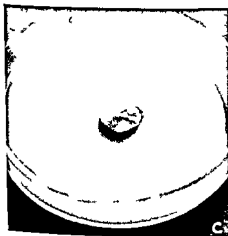
C Colony slow growing, attaining a diameter of 15 mm in fourteen days; deep coral in color, low convex, glistening, soft and smooth, occasionally becoming wrinkled.

D Microscopically, oval or round thin-walled budding cells.

> The genus consists of at least seven species showing a wide variation in shape and size of cell and in the number of buds (one to several) produced by a single cell, as well as in colony texture. They produce carotenoid pigments (red, orange or yellow). The cells form either no capsule or a faint one. No true mycelium or ascospores are produced.




a
b
a
b
B


C


c
D

MONILIA SITOPHILA
STREPTOMYCES SP.

Figure 31

Monilia sitophila

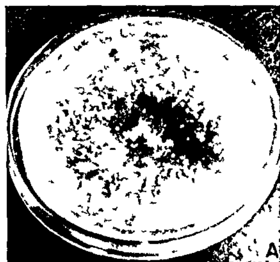
A. Colony rapidly growing, often completely filling the Petri plate or culture tube within three or four days; white at first, growing close to the surface of the agar, quickly becoming floccose and showing salmon colored masses.

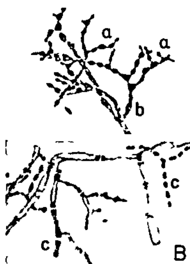
B. Microscopically: branching chains of ovate conidia (a) borne on short conidiophores (b); thick walled arthrospores (c) formed by breaking up of the old hyphae into separate cells.

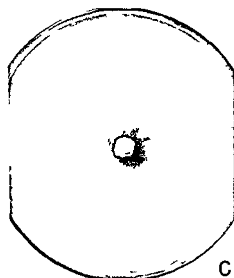
Streptomyces sp.

C. Colony slow growing, attaining a diameter of 6 mm. in eleven days; raised, slightly folded, hard, leathery, closely adherent to medium and covered with a white aerial mycelium. Many species are variously pigmented: white, gray, red, rose, lavender, green, depending largely on the nature of the media and the conditions of growth. Characteristically they produce a strong musty odor.

D. Microscopically: long, branching, aerial hyphae (a) of 1μ or less in diameter; from these hyphae arise spore bearing filaments (sporophores) which by segmentation form chains of spherical or oval spores (b). The sporophores may be straight, wavy, loosely or tightly spiraled.


A


a
a
b
c
c
B


C


b
a
a
D

FUSARIUM SP
CLADOSPORIUM (HORMODENDRUM) SP

Figure 32

Fusarium sp

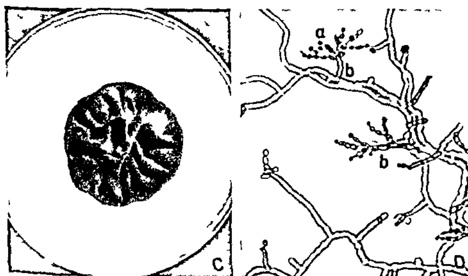
A Colony fast growing, attaining a diameter of 55 mm in seven days, at first white, later violet, with a center of heavy fluffy mycelium and a border of flat white mycelium. This genus consists of many species, some of which are of different colors: white, tan, pink, violet or red.

B Microscopically, branched or unbranched conidiophores (a) on thin hyphae; the conidiophores produce two types of spores: macroconidia long, multiseptate, sickle shaped (b); microconidia ovoid, one celled (c).

Cladosporium (Hormodendrum) sp

C *Colony moderately fast growing*, attaining a diameter of 42 mm in seventeen days, irregularly heaped and folded, with a smooth flat periphery; surface velvety, olive green; undersurface greenish black. This fungus does not grow at 37 C as do the pathogenic species of the same genus.

D Microscopically, mycelium olivaceous; conidia in branching tree-like chains (a) borne from branched conidiophores (b) of variable lengths.


a
b
b
C
D

PHOMA sp
PULLULARIA sp

Figure 33

Phoma sp

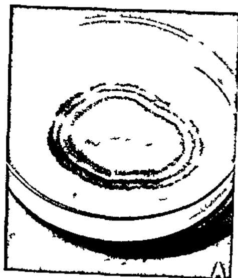
A. Colony fast growing, attaining a diameter of 70 mm. in twelve days, consisting of two distinct zones: a central umbonated zone (35-40 mm.) of gray to taupe aerial mycelium and a peripheral zone (14 mm. wide) of shorter napped brownish aerial hyphae with concentric darker slimy rings containing pycnidia; border flat, pinkish tan, having an outermost layer of thin cream to tan submerged hyphae. Reverse shows a smudged brownish black pigment at center with beige border.

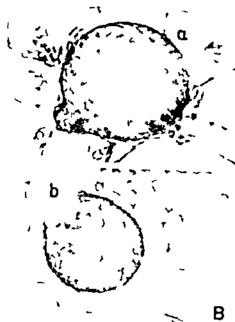
B. Microscopically, the identifying structures are the globose or flask-shaped pycnidia (a); upon crushing, these are found to be filled with many small ovate to elongate, one-celled hyaline pycnidiospores (b) that have been produced on short conidiophores inside the pycnidium. Some species of *Phoma* show pycnidia with one or more ostioles.

Pullularia sp

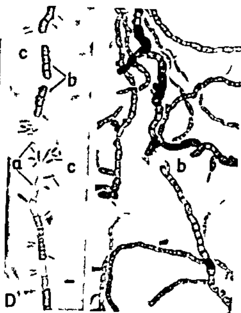
C. Colony fast growing, attaining a diameter of 60 mm. in fourteen days; at first white, flat and membranous, later showing an irregular, fairly raised, folded, black slimy center of yeastlike appearance surrounded by a flat greenish black border terminating in a fringe of greenish white mycelium.

D. Microscopically, hyphae of two types: hyaline, thin-walled (a) when young, later dark and thick-walled (b); conidia elliptical, sometimes budding (c), borne by repetition at various fertile points on mycelium.


A


a
b
B


C


c
b
a
c
b
D'

HELMINTHOSPORIUM SP
ALTERNARIA SP

Figure 34

Helminthosporium sp

A Colony moderately fast growing, attaining a diameter of 40 mm in fourteen days; grayish green, raised, with surface of heavily matted wooly mycelium.

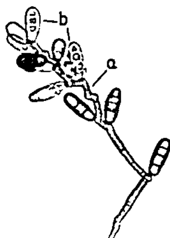
B Microscopically: hyphae light to dark, giving rise to long, septate, simple or branched conidiophores (a) having a knotted, twisted appearance; conidia brown, ovoid, containing four or more cells (b).

Alternaria sp

C Colony rapidly growing, attaining a diameter of 75 mm in nine days; grayish white at first, later greenish black; surface wooly or felt like with loose grayish mycelial tufts; border of grayish white mycelium.

D Microscopically: hyphae hyaline or dark; conidiophores (a) short or elongate, single or in groups, brown or greenish brown; conidia (b) brown, transversely and longitudinally septate (muriform), borne singly or in acropetal chains.

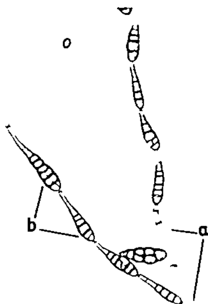




B

D





Aspergillus sp
Syncephalastrum sp

Figure 35

Aspergillus sp

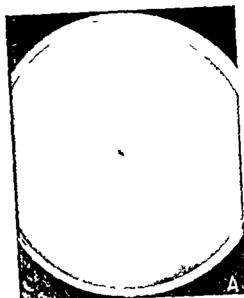
A Colony fast growing attaining a diameter of 70 mm in seven days flat with a center of thin grayish blue green somewhat floccose matted mycelium and border of sparse partially submerged grayish mycelium reverse pale yellowish green

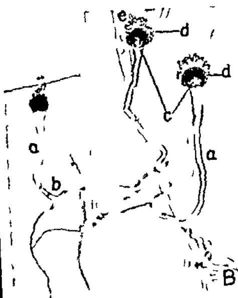
B Microscopically unbranched nonseptate conidiophore (a) arising from a specialized cell (b) in the mycelium (foot cell) at the apex of the conidiophore is the globose or elliptical vesicle (c) bearing phialides (d) (flask shaped structures) from which are produced chains of conidia (e)

Syncephalastrum sp

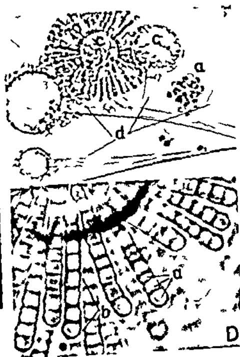
C Colony rapidly growing quickly filling the Petri plate with a dense white fluffy aerial mycelium later dark gray

D Microscopically mycelium nonseptate Sporangiospores (a) borne serially within finger like sporangioles (b) arising radially on inflated terminal ends (c) of branching sporangiophores (d)


A


e
d
d
c
a
a
b
B


C


c
c
a
d
a
b
D

Mucor sp
Rhizopus sp

Figure 36

Mucor sp

A Colony rapidly growing filling Petri dish with floccose aerial mycelium first white later gray

B C Microscopically mycelium nonseptate rhizoids absent sporangiophores (a) branched or unbranched borne at any point on the hyphae terminating in large globose many spored sporangia (b) sporangial wall easily ruptured scattering ellipsoid or globose spores (c) and revealing columella (d) (a swollen end of sporangiophore)

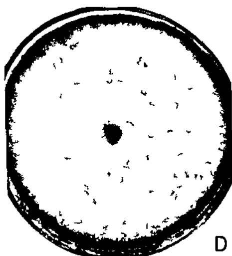
Rhizopus sp

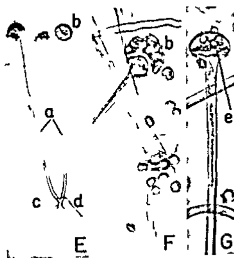
D Growth extremely rapid cottony grayish white aerial mycelium completely filling a Petri dish within three days

E C Microscopically mycelium nonseptate At the apex of a long erect unbranched sporangiophore (a) is borne the typical sporangium (b) a large spherical black spore case filled with many round brownish spores sporangiophores arise singly or in small groups directly opposite rhizoids (c) (root like structures) attached to the substrate and connected by hyphal branches (d) (stolons) running along the surface of the substrate The ruptured sporangium freed of spores reveals columella (e)


A


b
d
a
B
c
d
C


D


b
a
c
d
E
b
F
e
G

Media

Beef Infusion Broth with Glucose

Beef	450.0 gm
Peptone (Difco proteose)	20.0 gm
Sodium chloride	5.0 gm
Glucose	10.0 gm
Distilled water	1000.0 ml

Beef Infusion Agar with Glucose

Beef	450.0 gm
Distilled water	500.0 ml
Agar	17.5 gm
Peptone	10.0 gm
Sodium chloride	5.0 gm
Distilled water	500.0 ml
Glucose	10.0 gm

Beef Heart Infusion Agar with Blood

Beef heart	450.0 gm
Distilled water	500.0 ml
Agar	[illegible]0.0 gm
Sodium chloride	5.0 gm
Peptone	10.0 gm
Distilled water	500.0 ml
Horse, sheep, or rabbit blood, defibrinated, sterile	50 ml per 900 ml

Beef Infusion Agar with Blood and Antibiotics

Beef heart infusion agar or dehydrated blood agar base †	150.0 ml
Horse blood, defibrinated, sterile	10.0 ml
Penicillin (50 units per ml of medium)	8000.0 units
Streptomycin (50 units per ml of medium)	8000.0 units

Glucose Cystine Veal Infusion Agar †

Veal infusion	1000.0 ml
Peptone	10.0 gm
NaCl	5.0 gm
Agar	[illegible]0.0 gm
Cystine or cysteine hydrochloride	1.0 gm

Glucose Cystine Veal Infusion Agar (cont.)

Glucose 20% sterile solution	[illegible] ml
Blood (rabbit or horse)	50.0 ml

Combine and heat at 60° for three hours.

Potato Infusion Agar with Glucose

Potatoes, peeled and ground	200.0 gm
Distilled water	1000.0 ml
Agar	20.0 gm
Glucose	20.0 gm

Used unadjusted at pH 5.6-5.8 or adjusted subsequent to sterilization to pH 3.5 with a sterile 10% tartaric acid.

Corn Meal Agar

Agar	17.50 gm
Corn meal, yellow, ground	40.00 gm
Distilled water	1000.00 ml

Dissolve the agar in one half the water by autoclaving. Add the corn meal to the remainder of the water and heat at approximately 65° for one hour. Filter through paper. Admix the dissolved agar and the corn meal filtrate (500 + 500 ml.) and filter through cotton. Dispense as required and autoclave twenty minutes. No *pH adjustment is required.*

Czapek's Agar

$NaNO_3$	2.00 gm
K_2HPO_4	1.00 gm
$MgSO_4 \cdot 7H_2O$	0.50 gm
KCl	0.50 gm
$FeSO_4$	0.01 gm
Sucrose	30.00 gm
Agar	15.00 gm
Distilled water	1000.00 ml

pH 6.6

Rice Medium

Rice, white grain	[illegible]
Distilled water	[illegible] ml

Place rice and water in 300 ml flask with rice grains evenly distributed over bottom of flask. Set evenly in the sterilizer and autoclave twenty minutes.

—

Sabouraud's Glucose Broth

Bacto Peptone	10.0 gm
Glucose	40.0 gm
Distilled water to make	1000.0 ml

Final pH 5.[illegible]

Sabouraud's Glucose Agar [illegible]

Agar	20.0 gm
Peptone	10.0 gm
Glucose technical	40.0 gm
Distilled water to make	1000.0 ml

Adjusted subsequent to sterilization to pH 5.6-5.8 with sterile 10% tartaric acid

Sabouraud's Peptone Agar with Honey

Agar	
Peptone	[illegible]
Honey	[illegible]
Distilled water to make	[illegible]

Adjusted subsequent to sterilization to pH 5.6-5.8 with sterile 10% tartaric acid

Carbohydrate Broth for Fermentation Tests with Candida

Difco beef extract	
Sodium chloride	
Difco peptone	[illegible]
Distilled water	[illegible]

Heat to boiling and titrate exactly to pH 7.2. Add 100 ml of indicator solution, filter and dispense in 10-ml quantities in Durham type fermentation tube. Autoclave at 15 pounds pressure for exactly 15 minutes. Add 0.5 ml of a 20 per-cent solution of the carbohydrate. The broth should not be kept for more than two or three weeks because slight changes in pH may occur.

Indicator solution

Brom thymol blue	0.04 gm
Distilled water	100.00 ml

Add a small amount of 1N NaOH to make the solution alkaline. When indicator is in solution neutralize with 1N HCl until the exact neutral point is reached and one drop of either acid or alkali will cause a complete change of color.

(Martin, D. S., *et al.*, J. Bact., 34:99, 1937)

Wort Agar†

Agar	20.00 gm
Ammonium chloride, NH_4Cl	1.00 gm
Peptone	0.78 gm
Maltose, technical	12.75 gm
Malt extract	15.00 gm
Dextrin	2.75 gm
Glycerol	2.35 gm
Dipotassium phosphate, K_2HPO_4	1.00 gm
Distilled water	1000.00 ml

* Formulas are based on those given in Wadsworth, A. B.: Standard Methods of the Division of Laboratories and Research, New York State Department of Health, 3rd ed. Baltimore: Williams & Wilkins Co., 1947, chapter 13.

† This medium is available commercially in the dehydrated form.

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